

Ethanol production from supercritical-fluid-extrusion cooked sorghum

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is a starch-rich grain similar to maize (*Zea mays* L.), but sorghum has been underutilized for biobased products and bioenergy. This study was designed to investigate the effects of supercritical-fluid-extrusion (SCFX) of sorghum on ethanol production. Morphology, chemical composition, and thermal properties of extruded sorghum were characterized. Analysis of extruded sorghum showed increased measurable starch content, free sugar content, and high levels of gelatinized starch. SCFX cooked and non-extruded sorghum were further liquefied, saccharified, and fermented to ethanol by using *Saccharomyces cerevisiae*. The ethanol yield increased as sorghum concentration increased from 20 to 40% for both extruded and non-extruded sorghum. Ethanol yields from SCFX cooked sorghum were significantly greater than that from non-extruded sorghum (>5%).

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1. Introduction

The development of a global economy cannot depend solely on the finite reserves of petroleum. Concerns exist about a future that continues to be overly reliant on petroleum. The development of biobased products from renewable resource as an alternative to petroleum has been one of the approaches to address these concerns. From a government perspective, the goal for biobased industry is to provide at least 25% of the 1994 levels of organic carbon-based industrial feedstock chemicals

and 10% of liquid fuels by the year 2020, and to provide 50% of liquid fuels by the year 2050 (National Research Council, 2000). These targets have stimulated research to increase the use of biomass and to develop biobased products. Sugar sources, such as starch and cellulose are potential candidates for production of ethanol, biodiesel, and organic chemicals.

Grain sorghum (*Sorghum bicolor* (L.) Moench) is an important commodity crop for the semiarid regions of the world. In the United States, sorghum is one of the most important crops in the Midwest regions, and sorghum production ranks third among cereal crops. Sorghum is a starch-rich grain with similar starch content to maize. However, sorghum has been underutilized for biobased products and bioenergy. The major barriers to promoting sorghum utilization are the poor wet-milling

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properties and potentially lower digestibility of sorghum starch, which is about 90–95% that of corn (Leeson and Summers, 1997). Several factors have been suggested to be responsible for the low starch digestibility. First, some sorghum starch granules are imbedded in a protein matrix (Hoseney et al., 1974; Taylor et al., 1984) that could restrict starch gelatinization (Chandrashekar and Kirleis, 1988) and enzyme accessibility (Waniska et al., 1990). Second, a high content of dietary fiber in sorghum might decrease starch digestibility (Bach Knudsen et al., 1988) as there has been a highly negative correlation reported between starch digestibility and resistance starch formation. Third, the presence of phenolic compounds such as tannin in the sorghum might inhibit enzyme activity (Leeson and Summers, 1997). High levels of tannin, a polyphenol, can result as much as a 10% reduction in starch and protein digestibility (Ratnavathi and Sashidhar, 2000).

Pretreatment technologies have been developed to increase the bioconversion rate of cellulosic-based biomass into fermentable sugar. These include mechanical methods such as size reduction through milling and extrusion process; physical methods such as steaming, radiation, and sonication; chemical methods such as alkaline and acid hydrolysis; biological methods such as microbial and enzyme degradation; and a combination of these methods. Extrusion has been a widely used process. It differs from other food processing methods, in which several unit operations are performed simultaneously. In extrusion, the materials are subjected to heating, mixing, and shearing, resulting in physical and chemical changes during passage through the extruder. The major advantages of extrusion include increased digestible starch fraction, reduced molecular weight of biomolecules, creation of free sugars, changes in the native structure of biomolecules, and reduced viscosity of fermentation broth when using extruded products during fermentation (Linko et al., 1983; Camire and Camire, 1994; Govidasamy et al., 1995; Zhan et al., 2003a). Therefore, extrusion could be an effective process to improve the bioconversion rate of sorghum starch.

An innovative processing technology patented by Cornell University called the supercritical-fluid-extrusion (SCFX) process, discussed in detail by Rizvi and Mulvaney (1992), Rizvi et al. (1995), and Sokhey et al. (1996), combines the extrusion process and supercritical-fluid technology. The main difference between SCFX and conventional extrusion is the injection of supercritical carbon dioxide, which replaces water as the blowing agent for expansion during extrusion. This technology not only overcomes many of the limitations of the typical extrusion process, but also takes

advantage of the unique solvating power of supercritical-fluid in low-temperature extrusion processing. SCFX technology has been used for food processing applications, but it has not been used as a pretreatment method for the bioconversion process. The injection of supercritical-fluid carbon dioxide during extrusion process may break the intimate bonds between starch granules and the protein matrix and result in an increase in sorghum starch availability. The objectives of this research were to characterize the chemical compositions, morphology, and thermal properties of supercritical-fluid-extruded sorghum and to study the production of ethanol from SCFX cooked sorghum. Carbon dioxide is a major by-product from ethanol production. With more than 80% of marketable carbon dioxide used from sources other than ethanol plants, carbon dioxide is considered as a waste product (Anonymous, 2005). The increased utilization of carbon dioxide could help ethanol plants to reduce such emission and make a profit.

2. Materials and methods

2.1. Materials and extrusion

Sorghum sample was ground into powder (Cyclone Sample Mill, UDY Corp., Fort Collins, CO) with a particle size of less than 2 mm and used as control for ethanol fermentation. SCFX pellets from the same sorghum sample were provided by Wenger, Inc. (Sabetha, KS). Supercritical-fluid-extrusion was conducted at the Extrusion Research Laboratory in the Department of Food Science, Cornell University. The supercritical-fluid-extruder was designed with preconditioning and four heating zones. For preconditioning, the mixing cylinder speed, stream flow rate, and water flow rate were 199 rpm, 10 kg/h, and 20 kg/h, respectively. For extrusion, the temperature profile of 45-90-90-30 °C was used; the feed rate and extruder screw speed were 75 kg/h and 300 rpm, respectively; and the stream rate and carbon dioxide injection rate were 5 kg/h and 2 kg/h, respectively. The extruded pellets were milled (Cyclone Sample Mill, UDY Corp.) into powder with a particle size of less than 2 mm and were used as substrates for ethanol fermentation.

2.2. Chemical composition analysis

Starch content was measured using a commercial available kit (Megazyme, Ireland). Protein was determined via a nitrogen combustion method using a LECO Leco FP-528 Nitrogen Determinator (St. Joseph, MI) according to AACC method 46-30 crude

protein-combustion method (AACC, 2000). Nitrogen values were converted to protein content values by multiplying by 6.25. Pepsin digestibility was carried out as described in Mertz et al. (1984). Briefly, 200 mg of ground sorghum were mixed with 35 ml of pepsin (1.5 mg/ml) in 0.1 M phosphate buffer, pH 2.0. The mixture was placed in a water bath at 37 °C for 2 h with periodic vortexing. After 2 h in the water bath, the solutions were centrifuged and the supernatant decanted. The pellets were washed two times with 5 ml of 0.1 M phosphate buffer at pH 2.0. The residue was then dried overnight under vacuum and protein content measured via nitrogen combustion using a LECO instrument. Crude fiber, acid detergent fiber, and ash were determined using AOAC standard methods (AOAC, 1995). Free sugar was measured as glucose after liquefaction using the Lane and Eynon volumetric method (Plews, 1970).

2.3. Differential scanning calorimetry measurement

The thermal properties of non-extrusion cooked and SCFX cooked sorghum were studied with a differential scanning calorimetry (DSC) (DSC 7, Perkin-Elmer, Norwalk, CT), which was calibrated with indium and zinc standards before sample measurements. All measurements were conducted in a nitrogen atmosphere. Approximately 20 mg of ground sorghum was weighed into DSC pans (Ø5.5 mm × 2.3 mm), with approximately 60 mg of distilled water (three times the amount of sorghum (dry basis)) was added, and the pans were sealed. The samples were kept at 4 °C overnight before measurement at a temperature range from 30 to 130 °C with a heating rate of 10 °C/min. Enthalpy of the gelatinization and melting of the amylose–lipid complex were calculated from the area encompassed by the baseline and thermogram. The reported values are averages of two replicates.

2.4. Scanning electron microscopy

The ground sorghum was examined with a scanning electronic microscope (SEM) (Hitachi S-3500N, Hitachi Science System, Ltd., Japan) at an accelerated voltage of 5 kV. The surface was sputter coated with the mixture of 60% gold and 40% palladium particles before observation.

2.5. Fermentation and measurement of ethanol content

Saccharomyces cerevisiae (*S. cerevisiae*, ATCC 24860) was used for ethanol fermentation. Yeast cells

were maintained on YPD medium (per liter) with 20 g yeast extract, 5 g peptone, 5 g dextrose, and 20 g agar. Yeast cells were cultured in a rotary shaker with a shaker speed of 200 rpm at 30 °C for 48 h in a preculture media (2% glucose, 0.5% peptone, 0.3% yeast extract, 0.1% KH₂PO₄, and 0.05% MgSO₄·7H₂O (pH 5.5)). Termamyl 1201 (0.01 ml α-amylase/g dry starch) (Novozymes North America Inc., Franklinton, NC) was used for starch liquefaction. Amyloglucosidase solution (3000 U/ml) was used for starch saccharification based on 150 U/g dry-starch. Detailed methods for liquefaction and saccharification were described by Zhan et al. (2003b). Erlenmeyer flasks (250 ml) were used with 100 ml of fermentation medium containing (per liter): 3 g peptone, 1 g KH₂PO₄, and 1 g (NH₄)₂SO₄ at pH 3.8 and different substrate concentration of sorghum. The peptone and minerals were added as solids before liquefaction. The medium was inoculated with 6% yeast suspension (1 × 10⁶ cells/ml) and incubated in a rotary shaker (200 rpm) at 30 °C for 72 h.

Ethanol was obtained by distillation of the fermentation broth, and the concentration was determined according to the specific gravity method (AOAC-942.06, 1995). All experiments were replicated five times and the average values were reported. Fermentation efficiency was calculated as: (actual weight of alcohol produced/theoretical weight of alcohol produced from starch) × 100.

3. Results and discussion

3.1. Chemical compositions

Measurable starch content of SCFX cooked sorghum increased by 3.1%, compared with non-extruded sorghum (Table 1), indicating that extrusion did increase measurable starch. In addition, the free sugar contents, measured as reducing sugar were 11.5% for SCFX cooked sorghum, which was 41.6% greater than non-extruded sorghum, suggesting that molecular degradation of starch occurred during extrusion. Extrusion also increased protein digestibility of sorghum by about 8%. This is probably because that the large proteins may become dissociated into smaller subunits, and denaturation also exposes enzyme-susceptible sites, thereby extrusion improves protein digestibility (Ummadi et al., 1995). Hamaker (2004) reported that sorghum with high protein digestibility was shown to increase significantly in starch digestibility. The increase in protein digestibility must benefit the fermentation process.

It has been reported that extrusion increased the solubility of insoluble fiber (Camire, 1998). Extrusion most

Table 1
Chemical compositions of SCFX cooked and non-extrusion cooked sorghum

Treatment	Starch (%)	Protein (%)	Crude fiber (%)	Acid detergent fiber (%)	Free sugar (%)	Protein digestibility (% of protein)
Non-extruded	68.8	9.60	2.05	3.69	8.13	57.9
SCFX cooked	70.9	10.0	1.35	3.05	11.51	62.6
Composition change (%) ^a	3.1	4.2	−34.1	−21.0	41.6	8.1

^a Composition changes were calculated as: ((composition of SCFX cooked – composition of non-extruded)/(composition of non-extruded)) × 100.

likely solubilizes large molecules in a manner similar to that reported for starch (Camire, 1998). Our results showed that SCFX cooking significantly decreased the crude fiber and acid detergent fiber contents of sorghum (Table 1). In this study, crude fiber is the residue of insoluble sorghum left after extraction by dilute acid followed by dilute alkali. SCFX cooked sorghum showed a larger reduction in crude fiber, which is about 34.1% less than non-extrusion cooked sorghum. It was reported that the fiber presented in sorghum could negatively affect starch digestibility (Bach Knudsen et al., 1988). Sorghum with reduced fiber content could have high starch digestibility and could be favorable for ethanol fermentation. In addition, the reduction of acid detergent fiber indicates that some of cellulose was depolymerized into glucose during extrusion, which must contribute to the increase in digestible starch content, and consequently, increase the utilization of carbohydrates in the sorghum (Table 1).

3.2. Morphology of sorghum particles

Microstructures of non-extruded sorghum particles showed that most of the starch granules were surrounded by continuous protein matrix (Fig. 1A). On the other hand, SCFX cooked sorghum particles had porous structures with extensive cracks and holes on the particle surface (Fig. 1B), which could be caused by water and carbon dioxide expansion and evaporation during extrusion. Extrusion cooking applies mixing and shear forces as well as heat to sorghum, which could fragment the protein matrix that surrounds the starch granules and destroy the compact structure of starch granules (Fig. 1B), and consequently, may increase starch availability for fermentation. In addition to the water that was normally used in conventional extrusion, SCFX also utilized carbon dioxide, which has superior solvation power and could deeply penetrate and distribute throughout sorghum particles during extrusion. As the molten sorghum exits the die, the flash of carbon dioxide produces expanded extrudates with porous structures. Therefore, SCFX cooking could effectively damage the native structures of the sorghum, increase their surface

areas, and consequently, result in extrudates with more available starch for fermentation.

3.3. Thermal analysis

The thermograms of non-extrusion cooked sorghum showed two endothermic peaks with peak temperatures of 75.6 and 101.2 °C, respectively (Fig. 2; Table 2). The first thermo-transition was attributed to the gelatinization of sorghum starch, and the peak temperature was designated as the starch gelatinization temperature. The second melting-transition was thought to be a melting

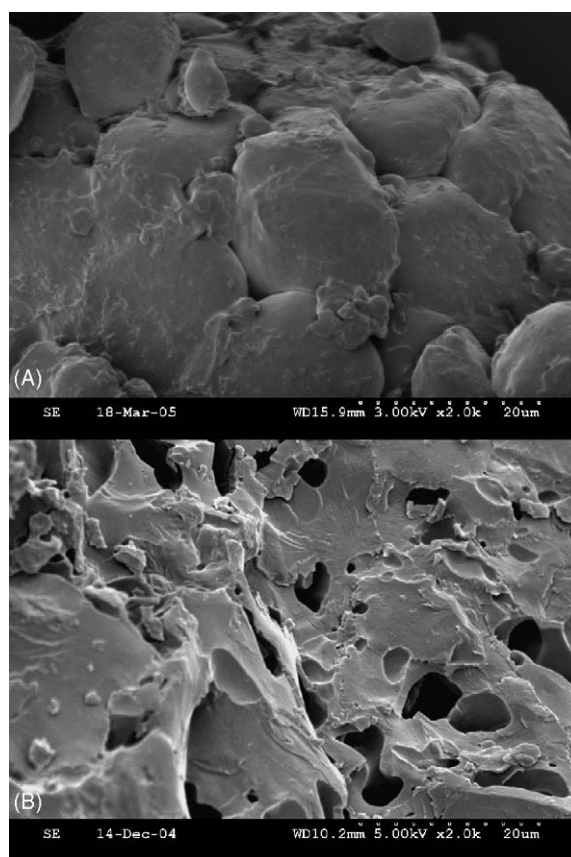


Fig. 1. Microstructures of sorghum: (A) non-extruded sorghum and (B) supercritical-fluid-extrusion cooked sorghum.

Table 2

Thermal analysis of SCFX cooked and non-extrusion cooked sorghum

Treatment	Starch gelatinization		Melting of amylose–lipid complexes	
	Peak temperature (°C)	Enthalpy (J/g)	Peak temperature (°C)	Enthalpy (J/g)
Non-extruded	75.65	5.512	101.2	0.478
SCFX cooked	58.08	0.541	113.5	1.117

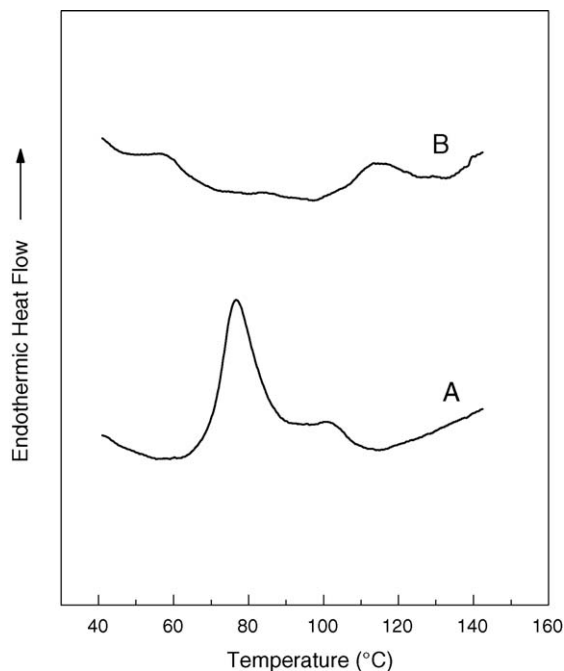


Fig. 2. Differential scanning calorimetry thermograms of sorghum: (A) non-extruded sorghum and (B) supercritical-fluid-extrusion cooked sorghum.

of starch–lipid complexes (Paton, 1987). The values of starch gelatinization temperature and enthalpy were in the range of reported values of 68–77 °C (Kelsall and Lyons, 1999) and 4.4–7.8 J/g (Aboubacar and Hamaker, 1999), respectively. Peak temperatures of SCFX cooked sorghum exhibited largely reduced starch gelatinization temperatures and enthalpies, compared with the non-

extruded sorghum (Table 2), indicating nearly complete gelatinization of starch in the extrudates. High values of starch gelatinization temperature and enthalpy usually indicate high thermal stability and a more ordered compact structure of starch (Miyoshi, 2002). Extrusion cooking destroyed the compact crystal structure of the starch and resulted in a much less ordered residue structure with low thermal stability. However, a small enthalpy value indicates that a few ordered structures possible remain after SCFX cooking. It should be noted, however, that the DSC measures only the amount of starch still in the crystalline state, but cannot measure the difference in starch availability due to protein disruption.

SCFX cooked sorghum also showed increased temperatures and enthalpies of melting of amylose–lipid complex (Table 2) suggesting the formation of new amylose–lipid complexes. Bhatnagar and Hanna (1994) reported similar observation of a helical complex formation when they extruded maize starch together with stearic acid. However, amylose–lipid complex would not reduce starch availability because it can be fully hydrolyzed by α -amylase (Seneviratne and Biliaderis, 1991). The use of α -amylase and amyloglucosidase during liquefaction and saccharification of sorghum would completely hydrolyze the complexes and release glucose for fermentation.

3.4. Ethanol production

Ethanol yields were in the range of 8.77–17.0% (v/v) for non-extruded and 9.14–17.87% (v/v) for SCFX cooked sorghum (Table 3). Ethanol yield increased

Table 3

Ethanol yields (% v/v) of SCFX cooked and non-extrusion cooked sorghum

Treatment	Sorghum concentration				
	20%	25%	30%	35%	40%
Non-extruded	8.77b ^a	10.72b	12.45b	14.74b	17.00b
SCFX cooked	9.14a	11.19a	13.05a	15.56a	17.87a
Increase in ethanol yield (%) ^b	4.22	4.38	4.82	5.56	5.12

^a Means of five replicates, based on least square differences (LSD) procedure at $\alpha = 0.05$ level, means with the same letter (a and b) in the same column are not significantly different.

^b Increase in ethanol yield was calculated as: ((ethanol from SCFX cooked – ethanol from non-extruded)/(ethanol from non-extruded)) \times 100.

Table 4
Fermentation efficiency of SCFX cooked and non-extrusion cooked sorghum

Treatment	Fermentation efficiency at varied sorghum concentration				
	20%	25%	30%	35%	40%
Non-extruded	87.87 ^a	85.93 ^b	83.17 ^b	84.39 ^b	85.16 ^b
SCFX cooked	91.57 ^a	89.68 ^a	87.17 ^a	89.08 ^a	89.52 ^a
Increase in efficiency (%) ^b	4.22	4.38	4.82	5.56	5.12

^a Means of five replicates, based on least square differences (LSD) procedure at $\alpha = 0.05$ level, means with the same letter (a and b) in the same column are not significantly different.

^b Increase in efficiency (%) was calculated as: ((efficiency from SCFX cooked – efficiency from non-extruded)/(efficiency from non-extruded)) \times 100.

as substrate concentration increased for both the non-extruded and extruded sorghum. At each substrate concentration, the SCFX cooked sorghum gave a greater ethanol yield than did the non-extruded sorghum. Ethanol yields from SCFX cooked sorghum were 4.2–5.6% greater than that from non-extrusion cooked sorghum with substrate concentrations from 20 to 40%. More than 5% increase in ethanol yield from SCFX cooked sorghum was achieved when the substrate concentrations of 35 and 40% were used.

Fermentation efficiency is normally used to indicate the efficiency of ethanol production. High fermentation efficiency means high starch conversion rate. The highest fermentation efficiency lies between 90 and 93% (Ingledew, 1999) because some glucose has to be used for production of cell mass, reactions of cell maintenance, and production of minor end metabolism products. Similar to the trend observed in ethanol yield, fermentation efficiency for SCFX cooked sorghum was greater than those for non-extruded sorghum, with SCFX cooked sorghum having the most efficiency of 90% (Table 4). Fermentation efficiency generally decreased as substrate concentration increased, however, suggesting the negative effects of a substrate with more solid content. The percentage increase of fermentation efficiency compared with non-extruded sorghum depended on sorghum concentration. The maximum value of percentage increase of fermentation efficiency was 5.56%, which was observed at 35% sorghum concentration (Table 4).

The extrusion process is an effective pretreatment method of sorghum for fermentation. Chemical changes (such as thermal degradation, depolymerization of starch, dietary fiber and proteins, and recombination of depolymerized fragments), and physicochemical changes (such as destruction of native starch and protein structures) could occur during the extrusion process (Camire, 1998). The protein matrix and disulfide bonds holding endosperm proteins together have been

reported to be responsible for reduced starch digestibility in sorghum (Chandrashekar and Kirleis, 1988; Zhang and Hamaker, 1998). Extrusion could break those protein bonds and disrupt the protein matrix, gelatinize starch, and make more starch available for enzyme hydrolysis, and consequently, increase ethanol yield and fermentation efficiency. In addition, extrusion could reduce the levels of phenolic compounds presented in sorghum known to be enzyme inhibitors as much as 50% (Zhan et al., 2003a). Extruded sorghum with reduced phenolic content could be broken more effectively during liquefaction and saccharification, resulting in an increased amount of glucose for ethanol fermentation.

4. Conclusions

Extrusion cooking increased ethanol yield and fermentation efficiency of sorghum. Improvements in the bioconversion of sorghum starch probably were from the release of starch from the protein matrix and enhancing the availability of starch for conversion to fermentable sugar. SCFX cooked sorghum showed greater fermentation efficiency than non-extrusion cooked sorghum and could be partly explained by an increased fermentable starch, decreased measurable fiber content, and fine porous structures produced by SCFX cooking. Both ethanol yield and fermentation efficiency were affected by sorghum concentration. Ethanol yield increased as substrate concentration increased, whereas greater fermentation efficiency was generally observed at a low substrate concentration. Finally, SCFX cooking is an effective pretreatment method that could improve the bioconversion rate of sorghum starch into ethanol.

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